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Scientific Research

Investigating the effect of nanoliposomes containing ascorbyl palmitate on the oxidative stability of soybean oil

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ABSTRACT

Ascorbyl palmitate is a fat-soluble ester of vitamin C and palmitic acid, which is used to increase the oxidative stability of oils. In this study, in order to increase its stability to the environmental conditions of heat, humidity, oxidation and light, encapsulation technology (nanoliposome) were used in refined soybean oil without antioxidants. In this regard, in this study, 5 concentrations of nanoliposomes containing ascorbyl palmitate (0, 50, 100, 200 and 500 ppm) were used to increase the oxidative stability of soybean oil stored at 63°C (electric oven) for a period of 16 days. Tests such as acidity, peroxide, conjugated dienes, anisidine and oxidative stability were performed on those oils. The results showed that acidity, conjugated dienes and anisidine increased with increasing storage time, but with increasing concentration of nanoliposome containing ascorbyl palmitate, the increase in these characteristics was less intense. By increasing the storage time until the 12th day, the amount of peroxide in the samples increased and then decreased, and with the increase in the antioxidant concentration, the amount of peroxide in the samples decreased. On the other hand, it was shown that increasing the concentration of nanoliposome from zero to 500 ppm in soybean oil increases the oxidative stability by about 96%. Finally, it was found that increasing the concentration of nanoliposomes containing ascorbyl palmitate in soybean oil reduces oil oxidation.

1- Introduction

The presence of free radicals in food causes oxidative spoilage, as a result of which, unsaturated fatty acids break down and form volatile compounds, and α-tocopherol in oil is lost during this process [1]. Antioxidants are food preservatives to increase the stability and shelf life of food products from the farm to the time of consumption against oxidative spoilage, and these compounds affect the acceptance of food by affecting its texture, color, taste, freshness, aroma and nutritional value [2]. Although today, efforts have been made to use more natural preservatives in food processing [3], but since synthetic antioxidants are cheap and available, and also have high stability and efficiency, they have received more attention. The most well-known synthetic antioxidants include butyl hydroxytoluene (BHT), butyl hydroxyanisole (BHA), tert-butyl hydroquinone (TBHQ) and propyl gallates (PG) [4], which are capable of increasing the shelf life of food by inhibiting the growth of microorganisms. They are pathogens and spoilage of food, as well as protection of food from damage caused by oxidative stress [5]. BHA and BHT are synthetic monohydric antioxidants that are highly soluble in fat and show good covering effects. Also, BHA has good thermal stability, but it creates a phenolic smell at high temperatures. For this reason, it is used in small quantities and together with other antioxidants. BHT is heat unstable and decomposes. Gallates (esters of gallic acid with alcohols) are destroyed in the thermal process under alkaline conditions and form a blue-black complex with iron in the presence of moisture. TBHQ is the best antioxidant to protect frying oils against oxidation, which is banned in Europe. The type and amount of permitted use of synthetic antioxidants are different according to the laws of different countries [6]. Ascorbyl palmitate (AP), a fat-soluble ester of vitamin C and palmitic acid, is a synthetic antioxidant approved to prevent the oxidation of oils [1]. Arabsorkhi et al. (2023) showed that the use of ascorbyl palmitate leads to an increase in the stability of oil stored at high temperatures [7]. Sometimes, in order to increase the stability of antioxidant compounds in relation to the environmental conditions of heat, humidity, oxidation and light, coating technologies are used. The use of liposomes containing these compounds improves the effectiveness of medicinal compounds and, as a result, increases the therapeutic effects and reduces their side effects. From a biological point of view, liposomes can be the carriers of some bioactive compounds because they are made from natural lipids and therefore are non-toxic and do not stimulate the immune system while having appropriate biocompatibility. Due to the retention of bioactive compounds inside the liposome, its stability can also increase [8]. Bojmehrani et al. (2022) and Ahmadi et al. (2022) showed that the microencapsulation of phenolic compounds using liposome leads to an increase in the antioxidant power of grape pomace and white tea extracts, which led to a decrease in the rate of oil oxidation [9 and 10]. The aim of this study was to investigate the effect of nanoliposomes containing the antioxidant ascorbyl palmitate on some oxidative parameters of soybean oil.

2- Materials and methods

2-1- Materials

For this research, refined soybean oil without antioxidants and lecithin was obtained from Khorasan cotton and oilseeds company, and other chemicals used such as sodium thiosulfate, NaoH, etc. were obtained from reputable companies.

2-2- Preparation of soybean oil containing ascorbyl palmitate nanoliposomes

In order to prepare nanoliposome containing ascorbyl palmitate, the method of Bojmehrani et al. (2022) was used, using a concentration of 1:7 ascorbyl palmitate to lecithin. Commercial lecithin obtained from soybean oil was added. In order to better combine this material and lecithin, this mixture was placed in a rotary dryer at a temperature of 50 degrees Celsius and under vacuum so that in addition to better mixing, some of its solvent will also evaporate. Above the phase transition, the liposomes were homogenized for 10 minutes. Then, the liposomal mixture was transferred to an ultrasonic probe (Hielscher, Germany) in an ice water bath (to avoid applying too much energy into the solution and lipid hydrolysis and oxidation) and 9 cycles of 20 seconds with 30 seconds of rest between cycles were applied to the samples. occurred. In this step, single-layered nanoliposomes were produced. Then these samples were dried by freeze drying (Christ α 1-4, Germany) for 48 hours [9]. After preparing nanoliposomes containing ascorbyl palmitate, 5 concentrations (0, 50, 100, 200 and 500 ppm) were prepared and added directly to soybean oil without antioxidants, and these oils were kept in an oven at 63°C for 16 days. Laboratory (Memert, Germany) were kept. After sampling in the intervals of 0, 4, 8, 12 and 16 days, the following tests were performed on them [9].

2-3- Measurement of acidity

AOCS Cd 3-63 (1993) method was used to determine acidity and the amount of acidity number was obtained from equation 1 [11].

Equation (1) $$A = \frac{2.82 \times V}{W}$$

In relation 1, V: the volume of consumed profit in milliliters, W is the weight of the sample in grams, A is the free fatty acids in terms of oleic acid in 100 grams of the sample.

2-4- Determining the peroxide

The amount of peroxide of the samples was obtained according to the method of AOCS Cd 8-53 (1993) and from equation 2 [11].

Equation (2) $$P = \frac{S \times M \times 100}{W}$$

In Equation 2, S is the consumption of sodium thiosulfate in milliliters, M is the molarity of sodium thiosulfate, W is the oil weight in grams, and P is the oil peroxide in meqO_2/kg.

2-5- Determining the conjugate dienone

In order to measure the conjugated dienone compounds, the oils were diluted with hexane at a ratio of 1:600 and their absorbance was measured at a wavelength of 234 nm using a spectrophotometer [12].

2-6- Determining the number of anisidine

The amount of anisidine in the oils was measured according to the method of AOCS Cd 18-90 (1993). For this purpose, 0.5 grams of oil in a 25 ml flask was made up to volume with isooctane, and then 5 ml of this solution was mixed with one ml of a 0.25% anisidine solution in Glycal acetic acid was mixed and after 10 minutes, its absorbance was read at a wavelength of 350 nm using a

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spectrophotometer. The amount of anisidine number was obtained from equation 3 [11].

Equation (3) $$A = \frac{25\times(1.2As-Ab)}{m}$$

In Equation 3, As and Ab respectively indicate the absorption of the solution before and after the reaction with anisidine solution, m and A also indicate the mass of the sample in grams and the anisidine number of the oil.

2-7- Determination of oxidative stability

In order to determine the stability of oils against oxidation, Ransimet (Metrohm, Switzerland) and AOCS Cd 12b-92 (1993) method were used, using a temperature of 110 degrees Celsius and an inlet air flow rate of 20 liters per hour [11].

2-8- Statistical analysis

To evaluate the effect of adding nanoliposomes containing ascorbyl palmitate on the properties of soybean oil, a completely random design of SAS software was used and Excel 2007 software was used to draw graphs.

3- Results and Discussion

3-1- The effect of nanoliposome containing ascorbyl palmitate on the acidity of soybean oil

The results shown in Figure 1 showed that with increasing storage time, the acidity of oils increased, and this increase was less severe in samples containing 500 ppm. On the other hand, with the increase in the concentration of nanoliposomes containing this antioxidant in soybean oil, the amount of acidity increased less. Keene et al. (2019), stated that in most samples, increasing the concentration of antioxidants decreases the intensity of the increase in acidity during the storage time, that the mechanism of different antioxidants may be different from each other, for example, some antioxidants through absorption of active oxygen or reaction They slow down oxidation with free radicals [13 and 14]. Bojmehrani et al. (2022) attributed the increase in acidity over time to the high storage temperature and the production of free fatty acids resulting from the breakdown of triglycerides in oils [9]. Yamani et al. (2022) also reported an increase in the acidity of olive oil with increasing storage time, which was in line with the results of this section [15].

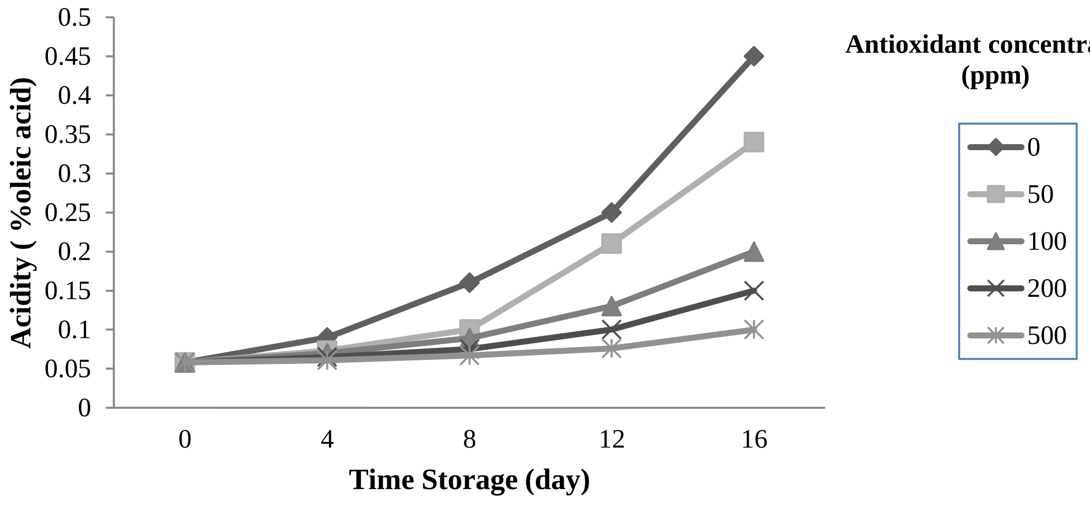

Figure 1- Effect of nanoliposome containing ascorbyl palmitate on oil acidity

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3-2- The effect of nanoliposome containing ascorbyl palmitate on oil peroxide

The results of comparing the averages of the peroxide value, which indicates the amount of hydroperoxides and is often considered as an indicator of the primary products of lipid oxidation [15], are given in Figure 2. As it is known, by increasing the storage time up to 12 days, the amount of peroxide in the samples increased and then decreased, and with the increase in the concentration of antioxidants in the oil, the increase in the amount of peroxide was less intense. The decrease in the amount of peroxide in the samples by increasing the storage time from 12 to 16 days can be attributed to the decomposition of hydroperoxides, secondary oxidation reactions and the production of carbonyls and volatile compounds [17]. Padehban et al. (2019) and Ettalibi et al. (2020) also stated that with increasing storage time, the amount of peroxide increased in olive oil and prickly pear seed, respectively, which was in accordance with the results of this section [18 and 19]. Phenolic compounds in antioxidants are able to give a hydrogen atom to free radicals and thus stop the chain reaction during the fat oxidation process [20]. The rate of formation of hydroperoxide is higher than its decomposition in the early stages of oxidation, but when the rate of decomposition of hydroperoxide exceeds the rate of its formation, the peroxide number can decrease even if oxidation increases during storage [21].

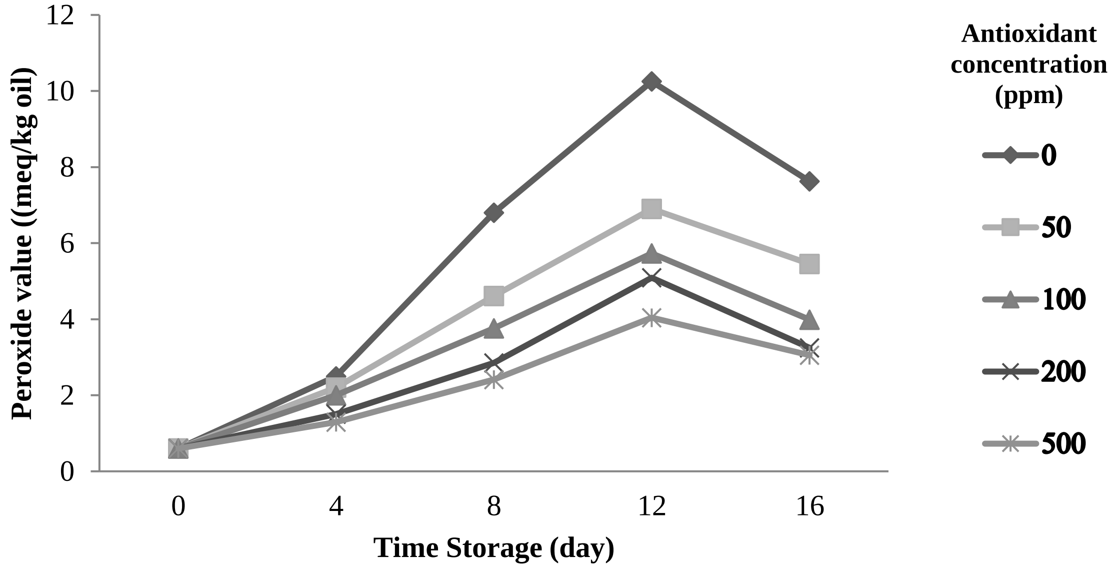

Figure 2- Effect of nanoliposome containing ascorbyl palmitate on oil peroxide

3-3- The effect of nanoliposome containing ascorbyl palmitate on conjugated dienone

Conjugated dienone is one of the products resulting from the primary oxidation of lipids, which is formed by the displacement of double bonds in unsaturated fatty acids and converts non-conjugated fatty acids into conjugated fatty acids [22]. The results showed that with increasing storage time, the conjugate dienone index increased, and the intensity of this increase, except for the sample containing 200 ppm nanoliposome containing ascorbyl palmitate, had a steeper slope in the last days, and also with the increase in the concentration of antioxidants in the oil, the amount of this index decreased, or in other words, in Higher concentrations of nanoliposome containing antioxidants had a milder slope of increasing the intensity of conjugated dienone index (Figure 3). Jafarpour et al. (2021) also stated in line with the results of this section that the increase in antioxidant concentration leads to the slowing down of the increase of conjugated dienone [23]. Conjugated dienone index is a good indicator of primary oxidation changes of lipids and antioxidants significantly prevent its formation. Of course, the type of oil also affects the rate of formation of conjugated dienone acids. The higher the oxygen intake, the higher the conjugated dienone index. On the other hand, the presence of phenolic and antioxidant compounds leads to a decrease in the progress of oxidation and isomerization of fatty acids, hence the amount of double dienone will increase less [24]. Some researchers considered the decomposition of hydroperoxides derived from linoleic acid by different forms of ascorbic acid as the reason for reducing the oil oxidation process [25].

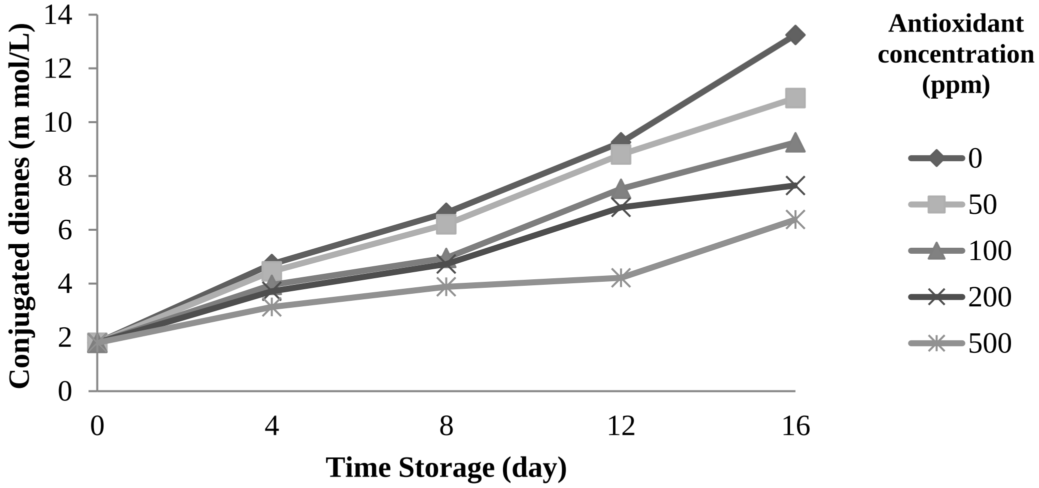

Figure 3- Effect of nanoliposome containing ascorbyl palmitate on oil conjugate dienes

3-4- The effect of nanoliposome containing ascorbyl palmitate on anisidine

Comparing the averages using Duncan's test method showed that anisidine index increased with increasing storage time, but this increase was less intense at higher antioxidant concentrations (Figure 4). The increase in anisidine number indicates the expansion of the spontaneous oxidation reaction and the increase of secondary products resulting from the decomposition of hydroperoxides and carbonyl compounds with the passage of time. There is a possibility that the suspended particles and to some extent the color change of the oil containing high concentrations of the mentioned extract can interfere in measuring the intensity of absorption at the wavelength of 350 nm and cause errors. Another possible reason for the results can be the Millard or pseudo-Millard reaction at high temperature, which reduces the effect of antioxidants during storage. On the other hand, these compounds absorb at a wavelength of 350 nm and cause an abnormal increase in absorption intensity [26]. The decrease of anisidine number with the increase of antioxidant concentration can be attributed to the increase of the antioxidant effect of these compounds. Some researchers stated that the use of natural antioxidants in oil leads to the reduction of anisidine number due to the reaction of these compounds with free radicals, which was consistent with the results of this section [27]. The reason for the decrease in anisidine number in the samples containing nanoliposome can be attributed to the greater protection of these compounds during the oven temperature, as well as the increase in the contact surface of these compounds to react with the free radicals in the oil [10].

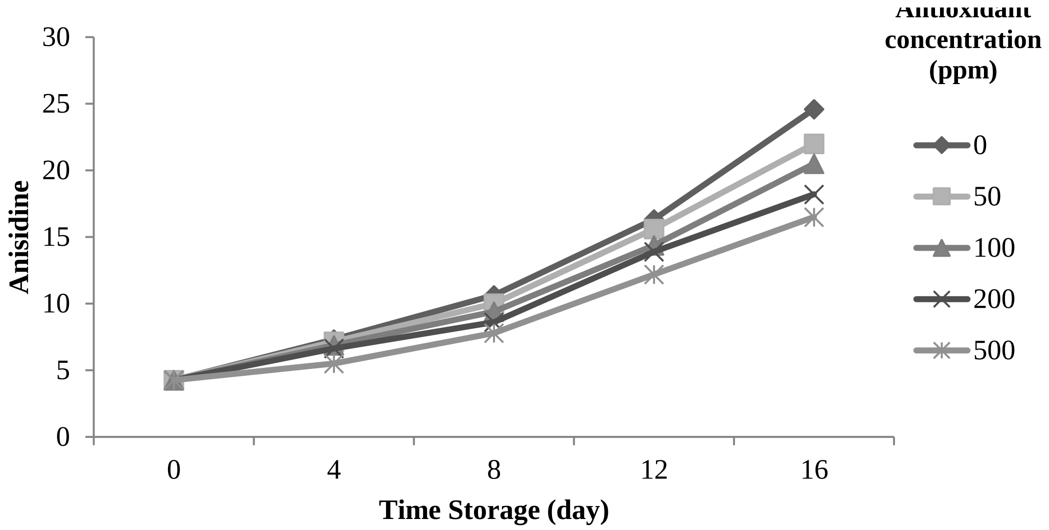

Figure 4- Effect of nanoliposome containing ascorbyl palmitate on oil anisidine

3-5- The effect of nanoliposome containing ascorbyl palmitate on oxidative stability

Oxidative stability is the length of time required to reach a point where one of the oxidation quantities such as peroxide number or carbonyl number suddenly increases after going through its increasing process and causes the production of unpleasant taste and smell in oil. Oxidation causes spoilage, which leads to an unpleasant smell and a decrease in food quality. There are several methods to evaluate the materials resulting from thermal processes that have many effects on the chemical, physical and nutritional properties of oil, one of the most important of which is the oxidation stability index [28]. Measuring the oxidative stability index during thermal processes of oils alone is not enough to evaluate the quality of oils, but it provides information about the initial state of the oil sample [29]. The results shown in Figure 5 showed that increasing the antioxidant concentration in the oil resulted in increasing the oxidative stability of the oil. In such a way that by increasing the antioxidant concentration from zero to 500 ppm, the oxidative stability increased by about 96%. Hosseini et al. (2021) also showed that increasing the antioxidant concentration due to the inhibition of oxidation increases the oxidative stability of the oil, which was consistent with the results of this section [2].

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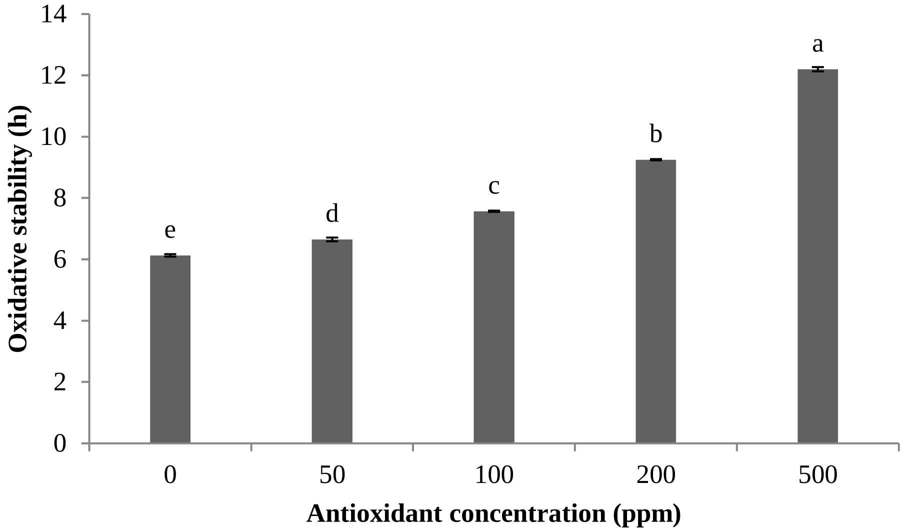

Figure 5- Effect of nanoliposome containing ascorbyl palmitate on oxidative stability

4- Conclusion

Since the dissolution rate of ascorbyl palmitate in oil is low, the main purpose of this study was to increase the oxidative stability of soybean oil using nanoliposome containing ascorbyl palmitate. According to the findings of this research, it can be said that increasing the storage time led to an increase in the acidity, conjugated dienone and anisidine of soybean oil, and on the other hand, with the increase in the concentration of nanoliposome containing ascorbyl palmitate, the increase in these characteristics decreased. Finally, it can be stated that increasing the concentration of nanoliposomes containing ascorbyl palmitate leads to increasing the oxidative stability of soybean oil.

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* مسئول مکاتبات:

چکیده

آسکوربیل پالمیتات یک استر محلول در چربی ویتامین C و اسید پالمتیک می‌باشد که از این ترکیب برای افزایش پایداری اکسایشی روغن‌ها استفاده می‌شود و در این مطالعه به‌منظور افزایش پایداری آن نسبت به شرایط محیطی حرارت، رطوبت، اکسایش و نور از فناوری درون پوشانی (نانولیپوزوم) در روغن سویا پالایش شده فاقد آنتی‌اکسیدان استفاده گردید. در این مطالعه از ۵ غلظت نانولیپوزوم حاوی آسکوربیل پالمیتات (۰، ۵۰، ۱۰۰، ۲۰۰ و ۵۰۰ پی‌پی‌ام) برای افزایش پایداری اکسایشی روغن سویا نگهداری شده در دمای ٦۳ درجه سانتی‌گراد (آون الکتریکی) برای مدت زمان ١٦ روز استفاده گردید و آزمون‌هایی از قبیل اسیدیته، پراکسید، دی‌ان مزدوج، آنیزیدین و پایداری اکسایشی روی آن روغن‌ها صورت پذیرفت. نتایج نشان داد که با افزایش زمان نگهداری میزان اسیدیته، دی‌ان مزدوج و آنیزیدین افزایش یافت ولی با افزایش غلظت نانولیپوزوم حاوی آسکوربیل پالمیتات روند افزایش این ویژگی‌ها از شدت کمتری برخوردار بود. با افزایش زمان نگهداری تا روز ۱۲ام، میزان پراکسید نمونه‌ها افزایش و سپس کاهش یافت و با افزایش غلظت آنتی‌اکسیدان میزان پراکسید نمونه‌ها کاهش یافت. از طرفی نشان داده شد که افزایش غلظت نانولیپوزوم از صفر تا ۵۰۰ پی‌پی‌ام در روغن سویا، میزان پایداری اکسایشی را در حدود ۹٦ درصد افزایش می‌دهد. در نهایت مشخص گردید که افزایش غلظت نانولیپوزوم‌های حاوی آسکوربیل پالمیتات در روغن سویا، اکسیداسیون روغن را کاهش می‌دهد.